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Ischemic Conditioning (Delay Phenomenon) Improves Esophagogastric Anastomotic Wound Healing in the Rat

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Background and Objectives: Esophagogastric anastomotic leaks are a major source of morbidity after esophagectomy. Occult ischemia of the mobilized gastric fundus is an important etiological factor for this failure of healing. To test the hypothesis that ischemic conditioning (delay phenomenon) could improve esophagogastric anastomotic healing, anastomotic healing was studied in a rodent model of partial gastric devascularization.

Methods: Thirty-four Sprague-Dawley rats (two groups of 17 rats) underwent partial gastric devascularization and creation of esophagogastric anastomoses. In the acute ischemia group, devascularization and anastomosis were done at the same laparotomy. In the ischemic conditioned group, devascularization was done 3 weeks before anastomosis. Gastric tissue perfusion was assessed by laser-Doppler flowmetry before and after devascularization in both groups, and 3 weeks after devascularization in the ischemic conditioned group. All rats were killed 4 days after anastomosis, and the wounds assessed for dehiscence, breaking strength, and hydroxyproline concentration.

Results: Gastric tissue perfusion, measured in tissue perfusion units (TPU) decreased immediately after devascularization (before: 73.6 ± 12.1 TPU; after: 25.0 ± 6.5 TPU; $P < 0.001$). After 3 weeks, gastric tissue perfusion returned to baseline values in the ischemic conditioned rats (before: 72.3 ± 11.0 TPU; 3 weeks, 71.1 ± 15.1 TPU; $P < 0.80$). Ischemic conditioned rats had fewer anastomotic leaks (2 vs. 9, $P < 0.023$) and higher anastomotic wound breaking strengths (2.35 ± 1.05 N vs. 1.56 ± .76 N, $P < 0.02$) than the acute ischemic rats. Anastomotic wound hydroxyproline concentration was not significantly different in the two groups (acute ischemic—0.111 ± .033 μmol/mg, ischemic conditions—0.097 ± .026 μmol/mg, $P < 0.20$).

Conclusions: In this rodent model of partial gastric devascularization, ischemic conditioning (delay phenomenon) ameliorated the harmful effect of ischemic on esophagogastric anastomotic wound healing.

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KEY WORDS: ischemia; gastrointestinal anastomoses; esophagectomy; delay phenomenon; surgical flaps; physiology

INTRODUCTION

Esophagectomy is the treatment of choice for many patients with esophageal cancer. Esophageal reconstruction is usually achieved by gastric pull up and esopha-

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gogastrostomy. Leakage of the esophagogastric anastomosis complicates 5–20% of procedures [1,2]. Esophagectomy for esophageal cancer has an operative mortality of approximately 10% [1]; between 30% and 50% of these deaths are the result of anastomotic leaks [3]. Elimination of anastomotic leaks would greatly reduce the morbidity and mortality of esophagectomy.

Mobilization of the stomach for gastric pull-up requires division of the left gastric, left gastroepiploic, and short gastric arteries. Because the gastroepiploic arcade is rarely complete, blood supply to the mobilized proximal stomach is largely derived from a rich submucosal plexus of vessels [4]. Although frank gangrene is rare if the stomach is properly mobilized [5], occult ischemia of the gastric fundus is often present [4,6]. Ischemia of the gastric fundus is a major factor in the etiology of esophagogastrostomy anastomotic leaks [2,7–9].

We have previously shown that gastric tissue perfusion gradually improves after acute gastric devascularization [10], and that this ischemic conditioning, or delay phenomenon, improves gastric wound healing in the rat [11]. Delay phenomenon is used in reconstructive surgery to improve the viability of flaps [12,13]. In some respects, mobilization of the stomach for esophageal replacement is analogous to flap transposition in plastic surgical reconstruction. To test the hypothesis that ischemic conditioning (delay phenomenon) could improve esophagogastric anastomotic healing, anastomotic healing was studied in a rodent model of partial gastric devascularization.

MATERIALS AND METHODS

Thirty-four female Sprague-Dawley rats (Charles River, Kingston, NY), weighing 200–250 g, were divided into two groups (acute ischemia and ischemic conditioned groups) of 17 rats each. The animals were housed three to a cage in conventional suspension cages, and allowed food and water until the time of surgery. Anesthesia was obtained with intraperitoneal pentobarbital sodium (Abbott Laboratories, Abbott Park, IL) (55 mg/kg), and a 3-cm midline laparotomy was made. Gastric tissue perfusion, at a point 3 mm distal to the esophageal-gastric junction, was measured by laser-Doppler flowmetry (Transonic BLF21, Ithaca, NY). Tissue perfusion was measured in tissue perfusion units (TPU) [14]. Partial gastric devascularization was done by ligating the left gastric artery. Gastric tissue perfusion was measured immediately after devascularization in both groups, and 3 weeks after devascularization in the ischemic conditioned group.

In the acute ischemia rats, the esophageal-gastric junction was then incised around 75% of its circumference. This left a small bridge of tissue posteriorly that simplified anastomotic suturing. The lower esophageal sphincter (an anatomic entity in the rat) was divided. The esophagogastric anastomosis was sutured with interrupted 7-0 polyprophylene sutures (Ethicon, Cincinnati, OH). In the ischemic conditioned group, esophagogastric division and anastomoses were done at a repeat laparotomy 3 weeks later. All laparotomy incisions were closed with a continuous 4-0 polyglactin 910 suture (Ethicon, Cincinnati, OH). Skin incisions were closed with the same suture, using a running horizontal mattress technique. Animals were allowed free access to food and water after recovery from anesthesia.

Animals not awakening from anesthesia for the first laparotomy were replaced, but thereafter animals that died were not replaced. All rats were killed 4 days after anastomosis by intraperitoneal pentobarbital sodium (120 mg/kg).

Esophagogastric anastomoses were assessed for dehiscence. Anastomoses were excised, and then incised longitudinally along their posterior aspect. Sutures were left in place. Anastomotic tissue strips were mounted in an Instron mini-44 tensiometer (Canton, MA) and distracted at 10 mm/min to measure breaking strength. Breaking strength was measured in Newtons (N). After that, anastomotic tissue (wound plus 1 mm of tissue on each side) was excised, weighed, and hydrolyzed in 6M HCL for 20 hours at 110°C. Following hydrolysis, the samples were spun, filtered through a 0.2 μm filter, diluted 1:50 and then subjected to hydroxyproline analysis [15].

Measured data are presented as means ± SD. Differences in tissue perfusion, breaking strength, and hydroxyproline concentration were assessed with Student's *t*-test. Anastomotic dehiscence rates were assessed with Fisher's exact test (two-tailed). A $P < 0.05$ was taken to be significant. The study was approved by the institutional Animal Care and Use Committee, and conducted in accordance with the National Research Council's guide for the care and use of laboratory animals.

RESULTS

Gastric tissue perfusion, measured in tissue perfusion units (TPU), decreased immediately after devascularization (before: 73.6 ± 12.1 TPU; after: 25.0 ± 6.5 TPU; $P < 0.001$). After 3 weeks, gastric tissue perfusion returned to baseline values in the ischemic conditioned rats (before: 72.3 ± 11.0 TPU; 3 week, 71.1 ± 15.1 TPU; $P < 0.80$). Ischemic conditioned rats had fewer anastomotic leaks (2 vs. 9, $P < 0.023$) and higher anastomotic wound breaking strengths (2.35 ± 1.05 N vs. 1.56 ± .76 N, $P < 0.02$) than the acute ischemic rats. Anastomotic wound hydroxyproline concentration was not significantly different in the two groups (acute ischemia—0.111 ± .033 μmol/mg, ischemic conditioned—0.097 ± .026 μmol/mg, $P < 0.20$).

Two rats in the ischemic conditioned group died, and were not replaced. One died of gastric necrosis 1 day after lapartomy and left gastric artery ligtaion. This rat's

gastric tissue perfusion was profoundly low after devascularization (<15 TPU). Another rat died of anesthetic causes at the time of its second laparatomy. Two rats in the acute ischemic group became very ill and were killed 1 day earlier than scheduled. Both had peritoneal sepsis secondary to anastomotic dehiscence.

DISCUSSION

The beneficial effects of delay phenomenon on flap vascularity and survival are widely known by plastic surgeons [12,13,16]. However, the physiological mechanisms of flap delay phenomenon are not completely understood [17]. Ischemic conditioning is one possible mechanism to explain the delay phenomenon. It is a process of neovascularization that occurs in response to ongoing ischemia within a tissue flap. Experimental studies support its importance in the delay process; the most ischemic parts of flaps show the greatest increase in vascularity over the delay period [18]. Another possible explanation for the beneficial effect of the delay phenomenon is altered microcirculatory flow from denervation [19,20]. Finally, tissue wounding in and of itself has an effect on tissue vascularity that is not specifically related to flap ischemia [21,22].

Ischemia is detrimental to esophagogastric anastomotic healing [2,8]. In previous animal studies gastric tissue perfusion gradually improved after devascularization [10]. The present experiment supports the hypothesis that ischemic conditioning, or delay phenomenon, improves esophagogastric anastomotic wound healing in the rat.

In clinical esophageal surgery, two-stage esophagectomy and reconstruction is occasionally performed in situations where the risk of esophagogastric anastomotic leakage is perceived to be very high. In this procedure, esophagectomy and gastric transposition is done at one operation, but creation of the anastomosis is delayed for 2–3 weeks. This is really an application of ischemic conditioning to clinical esophageal surgery. Although published experience with this two-stage procedure is limited, several investigators have noted a decrease in anastomotic leaks with its use [23,24].

Ischemic conditioning of the stomach could be clinically useful in reducing the incidence of leakage from esophagogastrostomy anastomoses. With modern minimally invasive surgical techniques, ischemic conditioning by partial gastric devascularization could be done laparoscopically. As surgeons gain more experience with pre-resectional laparoscopic staging of esophageal cancer [25,26], it is possible that laparoscopic ischemic conditioning of the stomach could be carried out at the time of laparoscopic staging.

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